Agriculture, Ecosystems and Environment 268 (2018) 52–60

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Elevated carbon dioxide and temperature imparted intrinsic drought tolerance in aerobic rice system through enhanced exopolysaccharide production and rhizospheric activation

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ARTICLE INFO

Keywords:
Elevated CO_2 and temperature
Aerobic rice
Water stress
Exopolysaccharide
Labile carbon
Plant enzymes

ABSTRACT

Elevation in atmospheric carbon dioxide (CO_2) concentration and temperature coupled with moisture stress is anticipated by most of the climate change prediction models (IPCC, 2014). Climate change results in atmospheric warming that trigger water stress to rice and could influence soil health, functioning and biological activities. Therefore, it is required to quantify indicative parameters like soil-exopolysaccharides which indicates greater water holding capacity of soil and imparted drought tolerance to rice. Carbon pools and related soil enzymes are also indicative of soil health status. We designed one field study in open top chambers (OTCs) to assess the impact of elevated CO_2, temperature and deficit moisture stress on rice. There were two replicated CO_2 enrichment treatment in OTCs, namely, ambient CO_2 (394 ± 20 ppm and ambient temperature; a-CO_2) and elevated CO_2 (550 ± 20 ppm) with temperature (2 °C ± 0.3 more than ambient; e-CO_2T). Three aerobic rice (grown in saturated soil moisture condition) cultivars (CR-143-2-2, APO and CR Dhan 201) were grown in separate blocks in each OTCs with adequate nutrient level and water stress (−40 kPa). Total soil and colloidal exopolysaccharides (EPSs), soil labile carbon (C) pools, soil enzymes (dehydrogenase, Fluorescein diacetate and β-glucosidase) and plant enzymes (catalase, peroxidase and super oxide dismutase) were measured as indicators of the soil health, functioning and intrinsic drought tolerance to the system. Total soil EPS (29%), colloidal EPS (37%), microbial biomass C (30%), readily mineralizable C (29%), dehydrogenase (15%), FDA (38%), and β-glucosidase (13%) activities were significantly higher under elevated CO_2 and temperature (e-CO_2T) to that of ambient condition (a-CO_2). The total and colloidal EPS, soil labile C pools and soil enzymatic activities were found higher at panicle initiation (PI) and grain filling (GF) stage than other physiological growth stages of rice. On the other hand, plant stress enzymes like catalase, peroxidise and superoxide dismutase (SOD) were decreased under e-CO_2T by 24, 20 and 32%, respectively, as compared to a-CO_2. All these indicated e-CO_2T could impart additional intrinsic drought tolerance to tropical aerobic rice system (aerobic rice cultivars grown with adequate nutrient supply) in future climate change scenario.

1. Introduction

The present atmospheric carbon dioxide (CO_2) concentration is about 394 μmol mol^{-1}. The concentration of CO_2 is anticipated to be 550 μmol mol^{-1} by 2050 owing to its current rate of increase of 1.9 μmol mol^{-1} y^{-1} (Solomon et al., 2007; IPCC 2007, 2014). Ongoing and projected changes in atmospheric CO_2 and other greenhouse gases may result in climatic anomalies related to temperature, precipitation, sea level rise, increase of extreme weather events, etc. (Lobell and Field, 2007; IPCC. Climate Change, 2014). Climatic anomalies, specifically elevated CO_2, temperature and moisture stress have direct consequences on agricultural productivity and more so on rice. As rice is a C_3 species and generally responds favourably to increased CO_2 concentration by increasing its carbon assimilation rate. However, its productivity is affected negatively with increase in temperature and more so when coupled with deficit moisture stress condition. (Cheng et al., 2010; Roy et al., 2012).

Deficit moisture stress is one of the major problems limiting rice productivity in the tropics, semiarid and arid regions of the world. Unseasonal drought in rice is a major consequence of climate change. It

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https://doi.org/10.1016/j.agee.2018.08.009
Received 16 April 2018; Received in revised form 28 June 2018; Accepted 8 August 2018
Available online 14 September 2018
0167-8809/

would deteriorate soil physico-chemical and biological properties which affect soil microbial activity and ultimately rice yield. Aerobic rice is one of the feasible options to address the deficit moisture stress situation of rice in tropics. It is the production system, where, rice is grown in aerobic soil condition (saturated soil moisture) like wheat and maize. However, aerobic rice in tropics is subjected to be grown under additional water stress exposed to elevated CO_2 and temperature (IPCC. Climate Change, 2014).

Additional water stress in soil could be partially overcome by increasing water holding capacity soil either through cultural practices or by microbial intervention. Water holding capacity of soil is improved by presence of exopolysaccharides (EPSs). It not only absorbs and retains moisture for longer time but at the same time improve soil aggregation which imparts intrinsic drought tolerance to system. Exopolysaccharides are high molecular weight, biodegradable polymers and biosynthesized by a wide range of bacteria (Vijayabaskar et al., 2011). Additionally, it protects the bacteria from environmental stresses (Iqbal et al., 2002). It protects bacterial cells from antibodies, bacteriophages and antimicrobial compounds. Moreover, hydrated EPS bacteria have 97% of water in polymer matrix that provides protection against desiccation (Bhaskar and Bhosle, 2005). High water retention capacity of exopolysaccharide helps in biofilm formation as well as protects soil from desiccation (Pereira et al., 2009; Di Pippo et al., 2011). Apart from this EPS also protect the soil microbes from UV radiation, enhances soil mechanical strength, antibiotic resistance and exo-enzymatic degradation activities (Pereira et al., 2009). It also plays an important role in rhizospheric carbon mobilization through water absorption/retention regulation.

Elevated CO_2, temperature and moisture stress are closely related to soil carbon pools and enzymes in rice rhizosphere (Bhattacharyya et al., 2013,2014,2016). Increasing atmospheric CO_2 could alter the labile soil carbon pools and carbon cycling in terrestrial ecosystem as well (Ainsworth and Rogers, 2007). Labile soil carbon pools including microbial biomass carbon (MBC) and readily mineralizable carbon (RMC) vary significantly under stressed environmental condition like water stress. The MBC and RMC are also identified as sensitive indicators of rhizospheric activities. Like carbon pools, soil enzyme activities are also influenced by elevated CO_2 and temperature in various rice growing stages (Bhattacharyya et al., 2013). Moreover, soil warming due to seasons and weather fluctuations could also increased enzyme activities (Sardans et al., 2008). Further, temperature alone or in combination with moisture influences the activities of soil enzymes. Soil enzymes like dehydrogenase (DHA), Fluorescein diacetate (FDA) and β-glucosidase (β-GLU) are effective ecological indicators which are quantifiable and sensitive to moisture stress, elevated CO_2 and temperature. These also indicate the rhizospheric activation and response of microbes to atmospheric / soil temperature and CO_2 change.

Based on above discussion the question arises how the elevated CO_2 and temperature along with deficit soil moisture stress affect the aerobic rice production system in tropics? Is anticipated elevated CO_2 and temperature would aggravated moisture deficit stress or provide additional tolerance to system to cope up with the situation? And how the soil health indicators like EPS, labile carbon pools and enzymatic activities behave in those anticipate climate change situation. So we hypothised that elevated CO_2 along with moisture stress could pose diverse impact on soil health and productivity of aerobic rice ecosystem. For testing the hypothesis, our objectives were, to study the performance of aerobic rice cultivars when subjected to additional moisture stress and exposed to elevated CO_2 and temperature, and to quantify rhizospheric EPS production, labile carbon pools and enzymatic activities under aerobic rice system.

2. Material and methods

2.1. Experimental site

Experimental site was at ICAR- NRRI in India, (20°25′N, 85°55′ E; 24 m s^2). It is in tropical climate with an average rainfall of 1500 mm. June to September receives around 70–80% of total precipitation. Elevated CO_2 and temperature condition was simulated under OTCs in field with two replications. The treatments (CO_2 concentration) were replicated twice in randomized block design (RBD) under corresponding OTCs (De Costa et al., 2006; Roy et al., 2012). Under each OTC the cultivars (CR 143-2-2, APO and CR Dhan 201) were grown in separate blocks (six blocks in each OTC; two blocks for each cultivar). The circular OTCs were having 4 × 3 m (diameter × height) dimension (Neogenesis Engineering Pvt Ltd, India). Ambient CO_2 (a-CO_2; 394 ± 20 µmol mol^{-1} CO_2); and chamber with elevated CO_2 and temperature (e-CO_2T; 550 ± 20 µmol mol^{-1} CO_2; 2 °C ± 0.3 higher temperature than ambient temperature) were maintained 24 × 7, throughout the crop growth period. The elevated temperature was maintained by putting infrared lamps (IR) inside the OTC. The IR lamps transfer heat to the soil and air above the surface without direct contact of a heating element on the soil (Roy et al., 2012). Two IR lamps (each of 1000 W output) in each e-CO_2T chamber was hanged 2.0 m above the soil surface. The operating wavelengths of the infrared lamps were above 1000 nm. The lamps were equipped with ceramic core coated with in coloy, a metal alloy effective in high temperature applications ensuring a consistent infrared wavelength. The on/off action of infrared lamps was controlled by power semi-conductor controllers operated by the program logic control (PLC; OTMATIC; M/s Magnetic Brains, Mumbai, Maharashtra, India).

2.2. Soil characteristics and crop-water management

The soil order was Aeric Endoaquept and the soil texture was sandy clay loam. There was non-significant variation in soil texture, bulk density, CEC and conductivity within the treatments. The soil separates varied across the treatments/samples were 24–26% clay, 20–22% silt and 53–55% of sand. The bulk density, CEC and electrical conductivity were varied between 1.41-1.43 Mg m^{-3}, 13.7–14.3 cmol (p^+) kg^{-1}, and 0.43–0.49 dS m^{-1} respectively in the soil samples. The total carbon and nitrogen content was 0.76 and 0.07%, respectively. Twenty one to twenty four days old seedlings of three aerobic rice cultivars (CR-143-2-2, APO and CR Dhan 201) were transplanted inside OTCs. Nitrogen application rate was 100 kg ha^{-1} and applied in 3 splits. The first nitrogen dose (40 kg ha^{-1}) was given at 7th day of transplanting. Next two splits were (30 kg ha^{-1}) at maximum tillering and panicle initiation. Basal dose of phosphorous and potassium were applied as single super phosphate and muriate of potash at a rate of 40 kg P_2O_5 ha^{-1} and 40 kg K_2O ha^{-1}, respectively. Rhizospheric soils were collected with sample probe nearer to root surface (within 2–5 cm from root) with replications under each variety and OTCs and stored in a polythene bag at room temperature for EPS analysis as well as refrigerator (4 °C) for microbial and bio-chemical (MBC, RMC, soil enzymatic essay) assays. Soil moisture potential was maintained at −40 ± 3 kPa lower than the recommended for aerobic rice (−30 kPa in sandy clay loam soil) (Belder et al., 2005) up to 10 days before harvesting of crops. Soil moisture tension was maintained by regulating field irrigation scheduling. Irrigation scheduling was based on the soil moisture characteristic curves (previously done for the experimental site) considering soil moisture tension (measured by tensiometer and pressure plate) and gravimetric moisture content of soil. Periodic checking of soil moisture content / tension was done during the study period. This was done with the hypothesis that anticipated climate change would cause addition water stress (limited) under elevated CO_2 and temperature condition. Although, it is expected higher moisture stress under e-CO_2T than a-CO_2, but for comparison and limited field replication of OTCs similar

water stress was employed to both the treatment in our study.

2.3. Total exopolysaccharide in soils

The phenol-sulphuric acid assay (Dubois et al., 1956) was used with a glucose standard to determine EPS amounts. Absorbance of EPS fractions was compared to the glucose standard curve to calculate µg glucose g^{-1} soil. In which 0.5 g of fresh soil was taken in a centrifuge tube, 5 ml 2.5 N HCl was added to it and was kept in boiling water bath for 3 h for hydrolysis. The solution was neutralized with the help of Na_2CO_3 (until CO_2 effervescence stopped). Then the volume was made up to 50 ml and centrifuge. The supernatant was collected, and 0.2 ml of collected supernatant was taken in a test tube and diluted up to 1 ml. After that 1 ml of 5% phenol sulphuric acid was added to it followed by 5 ml of 96% H_2SO_4. It was shaken and kept for 10 min and vortex for 30 s. It was again placed in water bath for 20 min. Finally the volume was made up to 10 ml. Then total carbohydrate was measured by phenol sulphuric acid method.

2.4. Colloidal exopolysaccharides

Colloidal exopolysaccharides was measured with 1 g of air dried soil samples. It was extracted by 10 ml of 100 mM EDTA which was added to1 g dry soil and centrifuged at 3600 rpm for 15 min. The aliquot was transferred to test tube and precipitated by dehydrated 70% cold 5 ml ethanol in splits. Then the test tube was kept in refrigerator overnight for precipitation. Precipitate then air dried slowly. Finally the weights of the precipitate and test tubes were taken to measure the amount of colloidal exopolysaccharides.

2.5. Exopolysaccharide producing bacteria in soil

Exopolysaccharide producing bacteria was isolated by using a selective media, Tryptic Soya Agar (TSA). It is also called as Tryptone Soya Agar or Soybean Casein Digest Agar. It is used for enumeration and maintenance of stock culture. The TSA media contain casein peptone (15.0 g l^{-1}), soya peptone (5.0 g l^{-1}), sodium chloride (5.0 g l^{-1}) and Agar (15.0 g l^{-1}). Casein peptone and Soya peptone provide nitrogen, vitamins and minerals. The final pH was maintained at 7.3 $\pm$ 0.2 at 25 °C. The sugars from soya peptone help in bacterial growth and sodium chloride maintains the osmotic balance. For isolation, 1 gm soil sample was taken and were diluted up to 10^{-4} dilution. Then 0.1 ml suspension from each diluted samples was taken and inoculated to TSA medium by spread plate technique and incubate for 24 h at 37 °C. After the incubation, the colonies were counted in 10^{-3} dilution and the log cfu was determined.

2.6. Soil labile carbon pools

Soil microbial biomass carbon (MBC) was estimated by modified chloroform fumigation-extraction method with fumigation at atmospheric pressure (Witt et al., 2000). The moist soils of 20 g from individual treatment were transferred into bottles. Each soil sample for fumigation received 2 ml of ethanol free chloroform. For fumigation, bottles were closed and agitated to spread the soil over the inner surface of the bottle, to mix the soil with the chloroform and to expose the maximum surface area to chloroform vapour and incubated for 24 h in dark at 25 °C. The soil was extracted with 80 ml of 0.5 M K_2SO_4 for 1 h. The filtrate was reacted with $K_2Cr_2O_7$ and titrated against 0.04 N ferrous ammonium sulfate (freshly prepared) using diphenylamine as an indicator. Readily mineralizable carbon (RMC), extracted with 0.5 M K_2SO_4 in soil samples was estimated (Inubushi et al., 1991) by wet digestion method (Vance et al., 1987). The fresh soil samples were extracted with 80 ml of 0.5 M K_2SO_4 solution (1:4 w/v, dry soil: K_2SO_4 extract) and shaken on an orbital shaker for 30 min. A 10 ml portion of the filtrate was digested with 2 ml of 0.4 N $K_2Cr_2O_7$ and 10 ml of concentrated H_2SO_4 for 30 min at 150 °C. All the samples and the blank were titrated against anhydrous 0.04 N ferrous ammonium sulfate (freshly prepared) using 3 to 4 drops of diphenylamine indicator. The RMC content was expressed in µg g^{-1} soil or mg kg^{-1} soil.

2.7. Soil enzymatic activities

Soil dehydrogenase enzymatic activity was done by reducing 2,3,5-triphenyl-tetrazolium chloride (Casida et al., 1964). In brief, 3 g of wet soil was taken in test tubes followed by the addition of 0.2 g of $CaCO_3$, 1 ml of 3% 2, 3, 5-triphenyl tetrazolium chloride (TTC) and 2.5 ml of distilled water. The whole contents were then vortexed and placed in incubator with stopper at 37 °C for 24 h. After 24 h the stoppers were removed and 10 ml of methanol was added to the contents and shake it for few seconds. A red color appeared due to the reduction of 2, 3, 5-triphenyl tetrazolium chloride into triphenyl formazan (TPF). The filtrate was diluted with methanol to 100 ml. The intensity of the color was measured by using a spectrophotometer at 485 nm. The dehydrogenase activity was expressed in µg TPF g^{-1} day^{-1}. Fluorescein diacetate (FDA) hydrolysis activity measurement was done by Schnürer and Rosswall (1982) method with the modification proposed by Adam and Duncan (2001). In brief, two grams of moist soil was placed in 50 ml conical flask and 15 ml of 60 mM potassium phosphate buffer (pH 7.6) was added. Stock solution of FDA (0.2 ml of 1000 µg FDA ml^{-1}) was added as substrate to start the reaction. The flasks were then stoppered and the contents were shaken by hand. The flasks were then incubated in an incubator at 30 °C for 20 min. After the period of incubation, 15 ml of chloroform/methanol (2:1 v/v) was added immediately to terminate the reaction. The intensity of yellow-green color of the filtrates was measured at 490 nm in a spectrophotometer. The FDA hydrolysis activity was expressed in µg fluorescein g^{-1} h^{-1}. The β-glucosidase activity was measured by following the protocol of Eivazi and Tabatabai (1988). One gm of moist soil was taken in a 50 ml Erlenmeyer flask. To it 0.25 ml toluene, 4 ml of modified universal buffer (MUB; pH 6.0) and 1 ml of p-nitrophenyl-β-D-glucoside (PNG; substrate of the reaction) was added. The flask was then swirled to mix the contents properly. The flask was then stoppered and incubated at 37 °C for 1 h. After 1 h the stopper was removed and 1 ml of 0.5 M $CaCl_2$ and 4 ml of Tris-(hydroxymethyl) aminomethane-sodium hydroxide (THAM; pH 12) were added to the contents to stop the reaction. The intensity of the yellow color was measured at 420 nm in a spectrophotometer. The β-glucosidase activity was expressed in µg p-nitrophenol g^{-1} h^{-1}.

2.8. Plant stress enzyme

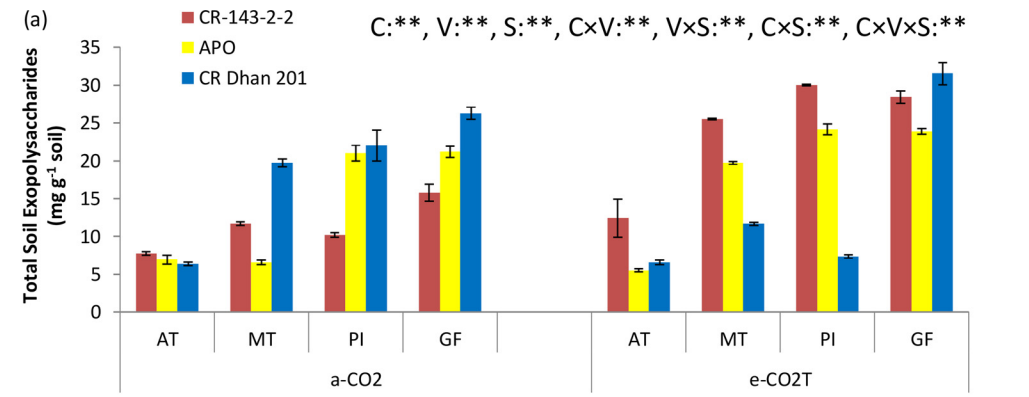
There were three plant stress enzymes (catalase, peroxidise and super oxide dismutase) were estimated under all the treatments. Catalase enzyme was estimated with the help of Beers and Sizer, (1952) method. Peroxidase activity was measured by the method of Hammerschmidt et al., 1982. Whereas, super oxide dismutase (SOD) was assayed by Dhindsa et al. (1981) method.

2.9. Yield

At maturity, rice plant were harvested manually and separated into grain and straw. The dry weight of straw was determined after oven drying at 50 °C to constant weight. The grain and straw yield at maturity were expressed as g m^{-2}.

2.10. Statistical analysis

The least significant difference at $p \leq 0.05$ level of each parameters at different rice growth stages was determined by using OPSTAT. SPSS 7.0 was used to determine the Pearson correlation matrix for soil labile carbon pools, total and colloidal EPS content, EPS producing bacterial



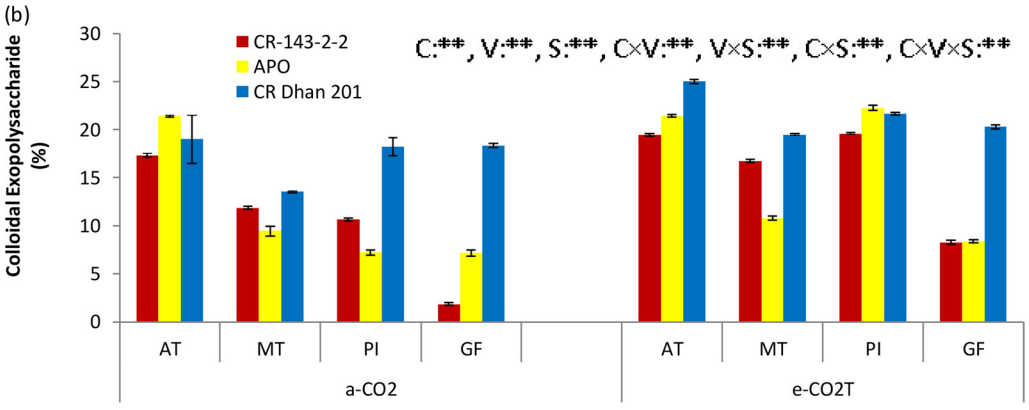


Fig. 1. Total soil exopolysaccharides production (a) and colloidal exopolysaccharide production (b) under ambient and elevated CO_2 and temperature condition in tropical flooded soil planted to rice (cv. CR-143-2-2, APO, CR Dhan 201) in various crop growth stages like AT (Active tillering), MT (Maximum tillering), PI (Panicle initiation) and GF (Green filling). Here, (**) represents significant level at 0.01 ($p \leq 0.01$). Here, C, V and S represent CO_2 concentration, variety and growth stages respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

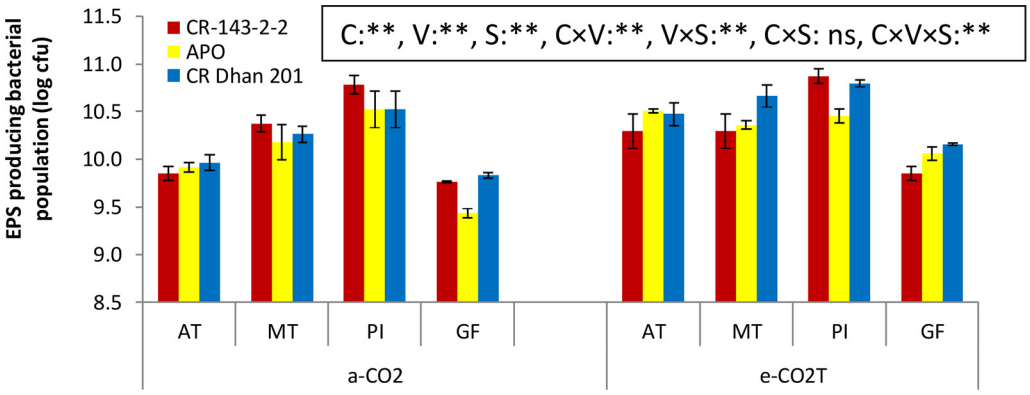


Fig. 2. Exopolysaccharides producing bacterial population under ambient and elevated CO_2 and temperature condition in tropical flooded soil planted to rice (cv. CR-143-2-2, APO, CR Dhan 201) in various crop growth stages like AT (Active tillering), MT (Maximum tillering), PI (Panicle initiation) and GF (Green filling). Here, (**) represents significant level at 0.01 ($p \leq 0.01$), ns: non-significant. Here, C, V and S represent CO_2 concentration, variety and growth stages respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

population and soil enzymatic activities. The individual effect of CO_2 concentration (ambient and elevated CO_2 and temperature), rice cultivars (three in number), stages of crop growth (four stage) and their interaction was studied. The ANOVA statistics in tables and figures are represented as delivered by Cheng et al. (2009).

3. Results

3.1. Total exopolysaccharide in soil

The total EPS was estimated under a-CO_2 and e-CO_2T in three aerobic rice cultivars at four critical physiological growth stages i.e. active tillering (AT), maximum tillering (MT), panicle initiation (PI) and grain filling (GF) (Fig. 1a). The average of total soil-EPS was higher under e-CO_2T (18.51 mg g^{-1} soil) than a-CO_2 (14.63 mg g^{-1} soil). Total soil-EPS was found higher at GF and was in the order of GF > PI > MT > AT. At GF, average total soil-EPS (pooled of three varieties) was also higher under e-CO_2T (27.92 mg g^{-1} soil) than a-CO_2 (21.07 $^{-1}$ soil). Among the cultivars, total EPS (average of all stages) was more under CR-143-2-2 (17.17 mg g^{-1} soil) followed by APO (16.42 mg g^{-1} soil) and CR Dhan 201 (16.10 mg g^{-1} soil). We found all the factors (CO_2T enrichment, cultivars and stages) and their interaction were significant. Total soil-EPS was significantly correlated with MBC (0.59**) and RMC (0.56**).

3.2. Colloidal exopolysaccharide

Overall, colloidal EPS content was in the range of 5.1–22% considering all the three varieties and four growth stages. The average of colloidal EPS content was found more under e-CO_2 T (17.8%) than a-CO_2 (13%). It was higher at AT than other stages. Among the three aerobic rice cultivars the average of colloidal EPS was more under CR Dhan 201 (19.4%) followed by APO (13.5%) and CR-143-2-2 (13.2%) (Fig. 1b), just opposite trend of total soil EPS.

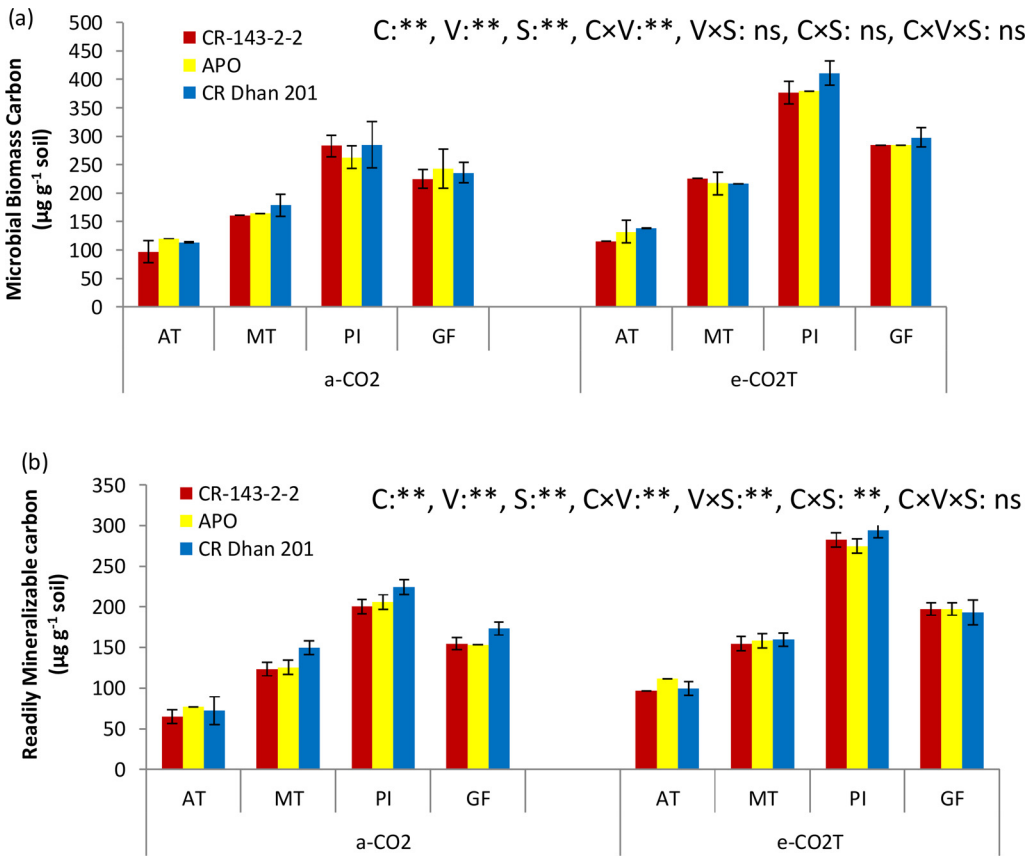


Fig. 3. Microbial biomass carbon (a) and readily mineralizable carbon (b) under ambient and elevated CO_2 and temperature condition in tropical flooded soil planted to rice (cv. CR-143-2-2, APO, CR Dhan 201) in various crop growth stages like AT (Active tillering), MT (Maximum tillering), PI (Panicle initiation) and GF (Green filling). Here, (**) represents significant level at 0.01 ($p \leq 0.01$), ns: non-significant. Here, C, V and S represent CO_2 concentration, variety and growth stages respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Exopolysaccharide producing bacterial population

Exopolysaccharide producing bacteria were enhanced under e-CO_2T but varying under rhizosphere of three cultivars at four growth stages (Fig. 2). On average the EPS producing bacterial population at four growth stages was higher under e-CO_2T (10.4 log cfu) than a-CO_2 (10.1 log cfu). However, among the three rice cultivars, EPS producing bacterial population (average of all stages) was more under CR Dhan 201 (10.33 log cfu) followed by CR-143-2-2 (10.26 log cfu) and APO (10.18 log cfu). EPS producing bacterial population was significantly correlated with RMC (0.42*), colloidal EPS content (0.43*) and all the three soil enzymes, i.e. FDA (0.56**), DHA (0.75**) and β-glucosidase (0.64**) and represented in Table 2.

3.4. Soil labile carbon pools

Soil microbial biomass carbon is the key indicator of biological activity of soils. And changes in MBC make influences on labile carbon pools and rhizospheric activity. Under a-CO_2 and e-CO_2T at different rice growth stages, MBC was ranged between 97.2–410.9 µg g^{-1} soil (Fig. 3a). The average (stages and cultivars) MBC content was higher under e-CO_2T (256.6 µg g^{-1} soil) as compared to a-CO_2 (197.7 µg g^{-1} soil). It was also varied significantly in rhizosphere of aerobic rice cultivars. It was higher under CR Dhan 201 (234.6 µg g^{-1} soil) followed by APO (225.6 µg g^{-1} soil) and CR-143-2-2 (221.2 µg g^{-1} soil) (average over the stages).

Readily mineralizable carbon was higher under e-CO_2T (185 µg g^{-1} soil) as compared to a-CO_2 (143.7 µg g^{-1} soil) (average over all the stages and cultivars; Fig. 3b). RMC content was higher at PI stage than other critical growth stages and was in the order of PI > MT > GF > AT. Unlike MBC, among all the three aerobic rice varieties, average RMC content was more under CR-143-2-2 (170.8 µg g^{-1} soil) followed by APO (162.9 µg g^{-1} soil) and CR Dhan 201 (159.3 µg g^{-1} soil).

3.5. Soil enzymatic activities

Dehydrogenase activities are considered as the most sensitive indicator of soil respiration. Under a-CO_2 and e-CO_2T, DHA activity was in the range of 82.61–424.19 µg TPF g^{-1} day^{-1} (Fig. 4a). Overall the average of all stages, it was observed higher under e-CO_2T (287.68 µg TPF g^{-1} day^{-1}) than a-CO_2 (251.15 µg TPF g^{-1} day^{-1}). However, among the three rice cultivars, DHA activity (average of all stages) was more under CR Dhan 201 (298.43 µg TPF g^{-1} day^{-1}) followed by APO (255.98 µg TPF g^{-1} day^{-1}) and CR-143-2-2 (253.84 µg TPF g^{-1} day^{-1}). Interaction effect on stages, varieties and CO_2 elevation on DHA was significant.

The FDA activity was also higher at PI stage as compared to other growth stages under a-CO_2 and e-CO_2T as well as varieties and it was in the order of PI > GF > MT > AT. Average (over stages and varieties) FDA activity was significantly higher under e-CO_2T (8.18 µg fluorescein g^{-1} h^{-1}) than a-CO_2 (5.92 µg fluorescein g^{-1} h^{-1}) (Fig. 4b). Average (over stages and CO_2 elevation), the FDA activity was more in CR Dhan 201 (7.64 µg fluorescein g^{-1} h^{-1}) rhizosphere compared to APO (6.81 µg fluorescein g^{-1} h^{-1}) and CR-143-2-2 (6.7 µg fluorescein g^{-1} h^{-1}).

β-glucosidase activity in rice rhizosphere was varied in the range of 38.36–78.76 µg p-nitrophenol g^{-1} h^{-1}. It was higher under e-CO_2T (59.05 µg p-nitrophenol g^{-1} h^{-1}) than a-CO_2 (52.4 µg p-nitrophenol g^{-1} h^{-1}). Like FDA, it was found more at PI stage (Fig. 4c). Varietal difference was also observed. Among the three aerobic rice cultivars, β-glucosidase activity (average of all stages) was highest under APO (57.7 µg p-nitrophenol g^{-1} h^{-1}) followed by CR Dhan 201 (57.1 µg p-nitrophenol g^{-1} h^{-1}) and CR-143-2-2 (52.3 µg p-nitrophenol g^{-1} h^{-1}). Interaction effect as well as main effect of stages, cultivars and CO_2 + temperature elevation was statistically significant.

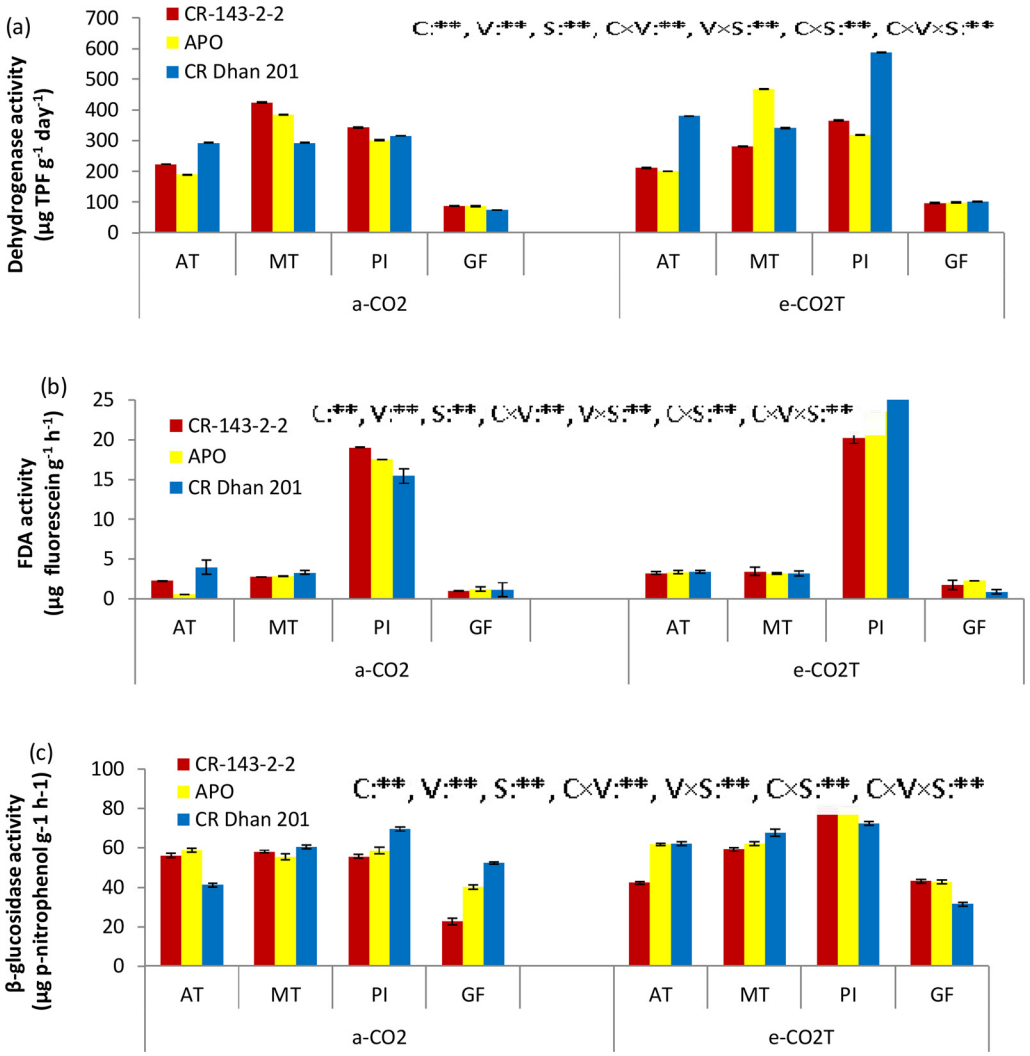


Fig. 4. Dehydrogenase (a) FDA hydrolysis (b) and β-glucosidase (c) activity under ambient and elevated CO_2 and temperature condition in tropical flooded soil planted to rice (cv. CR-143-2-2, APO, CR Dhan 201) in various crop growth stages like AT (Active tillering), MT (Maximum tillering), PI (Panicle initiation) and GF (Green filling). Here, (**) represents significant level at 0.01 ($p \leq 0.01$). Here, C, V and S represent CO_2 concentration, variety and growth stages respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.6. Plant stress enzymes

Three plant stress enzymes (catalase, peroxidase and super oxide dismutase) were estimated under a-CO_2 and e-CO_2T of three aerobic rice cultivars at two most critical rice growth stages i.e. MT and PI (Fig. 5a–c). It was observed that the enzymes were more under a-CO_2 as compared to e-CO_2T. The average (varieties and stages) catalase, peroxidase and SOD activities were 31, 24 and 46% higher under a-CO_2 than e-CO_2T. However, within three aerobic rice varieties, plant stress enzymatic activities varied non-significantly.

3.7. Grain and straw yield

Grain and straw yield of the rice cultivars were higher under e-CO_2T as compared to a-CO_2 although water stress was provided to both of them (Table 1). Among all the rice cultivars, higher grain yield was observed in CR Dhan 201 (375.7 g m^{-2}) followed by CR-143-2-2 (365.2 g m^{-2}) and APO (334.15 g m^{-2}) under a-CO_2, whereas under e-CO_2T, grain yield was higher in CR Dhan 201 (488.95 g m^{-2}) followed by APO (475.85 g m^{-2}) and CR-143-2-2 (461.31 g m^{-2}). The average grain and straw yield were less in a-CO_2 than that of potential yield as additional moisture stress was applied to aerobic rice cultivars. However, under e-CO_2T, it was recovered partially.

4. Discussion

4.1. Total and colloidal EPS production

We found total as well as colloidal EPSs contents in soil were increased significantly under elevated CO_2 and temperature than ambient. Those primarily due to significant increase in rice root biomass, higher root exudation (leads to enhanced C allocation in belowground) and higher microbial activities in rice rhizospheric under elevated CO_2 and temperature (Roy et al., 2012; Bhattacharyya et al., 2013, 2014). Under CO_2 enrichment freshly added higher amount of root-derived carbon into soil stimulates SOC decomposition in rice rhizosphere (Cheng et al., 2010), which subsequently release more dissolved carbon into soil and aggravates microbial and enzymatic activities. Elevated CO_2 up to certain extent (in case of our study, 550 ppm) also increased the plant production of C_3 plants like rice (Roy et al., 2012). Higher root biomass and root exudation under e-CO_2T also alters structural functional diversities in soil microbes (Bhattacharyya et al., 2013, 2014). We got higher colloidal exopolysaccharides of microbial origin under elevated CO_2T and are more so in moisture stress condition. That might

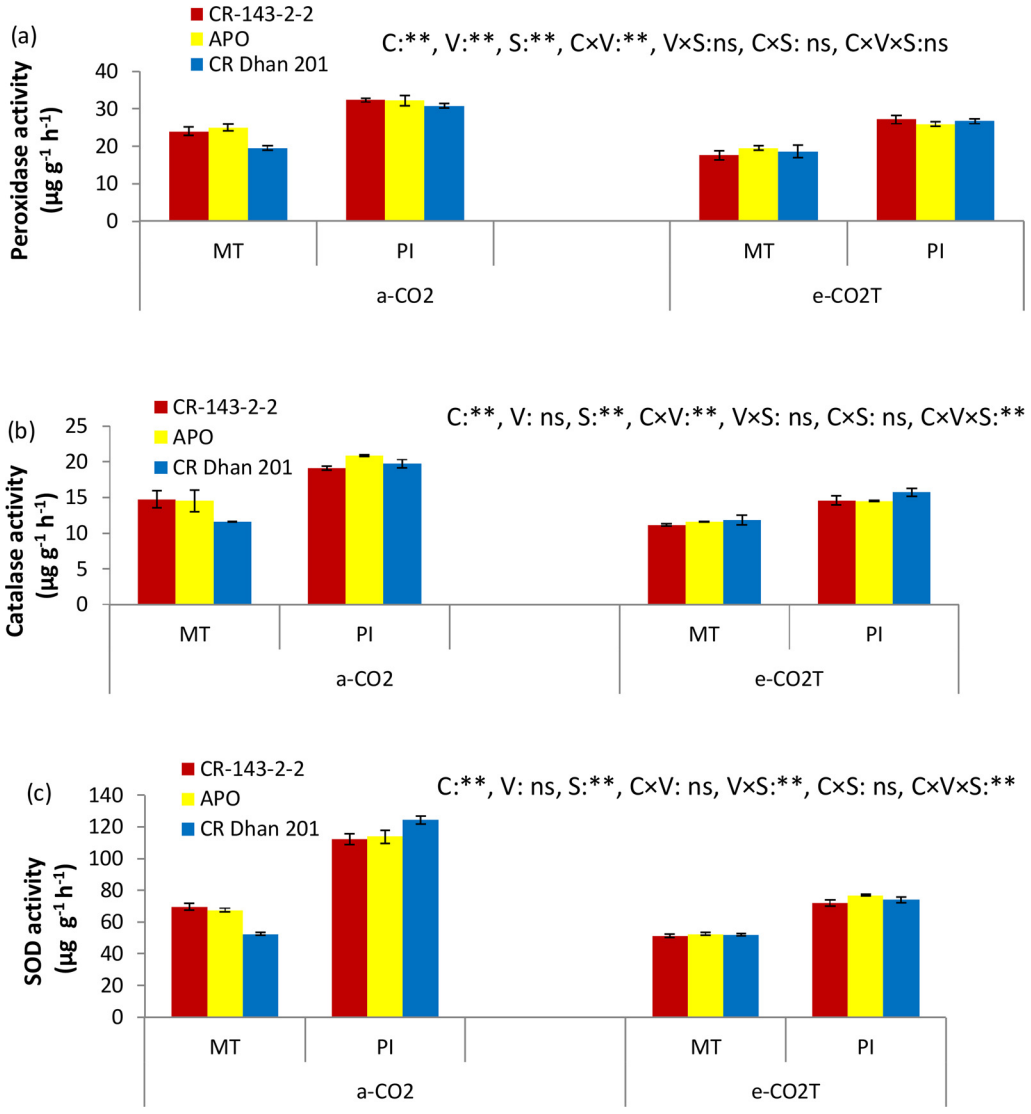


Fig. 5. Peroxidase (a), Catalase (b) and SOD (c) activity under ambient and elevated CO_2 and temperature condition in tropical flooded soil planted to rice (cv. CR-143-2-2, APO, CR Dhan 201) in various crop growth stages like AT (Active tillering), MT (Maximum tillering), PI (Panicle initiation) and GF (Green filling). Here, (**) represents significant level at 0.01 ($p \leq 0.01$), ns: non-significant. Here, C, V and S represent CO_2 concentration, variety and growth stages respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Grain and straw yield under a-CO_2 and e-CO_2T of three aerobic rice cultivars (CR-143-2-2, APO and CR Dhan 201). Here, C = CO_2 treatment (a-CO_2 and e-CO_2T)Fig; V = Variety.

Treatments		Grain yield ($g\,m^{-2}$)			Straw Yield ($g\,m^{-2}$)		
a-CO_2	CR-143-2-2	365.2 ± 7			554.1 ± 7		
	APO	334.2 ± 7			543.7 ± 12		
	CR Dhan 201	375.7 ± 12			544.7 ± 5		
e-CO_2T	CR-143-2-2	461.3 ± 7			685.4 ± 7		
	APO	475.9 ± 7			751.6 ± 3		
	CR Dhan 201	488.9 ± 5			657.6 ± 11		
ANOVA Statistics		C	V	C × V	C	V	C × V
	Error df	12	12	12	12	12	12
	Calculated F	992	19	13	1516	49	57
	Significance level	**	**	**	**	**	**

increased the moisture retention capacity of soil in our case as it is commonly known to increase water holding capacity of soil and particularly in biological soil crust (BSC) covered soils (Belnap et al., 2008). It has been also postulated that exopolysaccharides reduce

Table 2
Correlation matrix for soil labile carbon pools, total and colloidal exopolysaccharide content, exopolysaccharide producing bacterial population and soil enzymatic activity.

	MBC	RMC	TEPS	CEPS	EPSP	FDA	DHA	BGLU
MBC	1							
RMC	0.98**	1						
TEPS	0.59**	0.56**	1					
CEPS	NS	NS	NS	1				
EPSP	0.31	0.42*	NS	0.43*	1			
FDA	0.75**	0.79**	0.13	0.09	0.56**	1		
DHA	0.17	0.25	NS	0.28	0.75**	0.41*	1	
BGLU	0.32	0.41*	NS	0.52**	0.64**	0.51*	0.69**	1

MBC = Microbial biomass carbon, RMC = readily mineralizable carbon, TEPS = total soil exopolysaccharide content, CEPS = colloidal exopolysaccharide content, EPSP = exopolysaccharide producing bacterial population, FDA = Fluorescein diacetate assay, DHA = Dehydrogenase activity, BGLU = β-glucosidase activity.

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

infiltration in sandy soil by clogging soil pores. Elevated CO_2 could improve photosynthesis efficiency in rice by uncoupling energy demands and carbon concentrating mechanisms that subsequently enhance exopolysaccharide production in soil (Engel, 2002). Hence, EPS producing bacteria were enhanced which could lead higher water holding capacity of rhizosphere soil that helps to survive the plants under limited water stress condition. Therefore, total EPS in soil and colloidal EPS (mostly from microbial origin) could be effectively used as indicator in addition drought tolerance mechanism in aerobic rice in anticipated climate change scenario.

4.2. Soil labile carbon pools

Elevated CO_2 concentration facilitates rhizodeposition and influenced rhizospheric carbon dynamics and soil labile carbon pools (Bhattacharyya et al., 2013). In this study, soil labile carbon pools like, MBC and RMC increased under e-CO_2T because of increased accumulation of rhizodeposits and enhanced microbial growth and activities (Bhattacharyya et al., 2016). Apart from regulating soil nutrients MBC also plays a key role in increasing water holding capacity of soil (by soil aggregation) and nutrient cycling (He et al., 2003; Roldan et al., 2006). And it also recognised as sensitive indicator for soil health assay which is sensitive to elevated CO_2 and temperature (Bhattacharyya et al., 2013). We got higher soil carbon in rice rhizosphere under e-CO_2T due to higher microbial activities and root exudation which not only regulated and enhanced water availability, but, also imparted intrinsic drought tolerance to aerobic rice system. Intrinsic drought tolerance might be indirectly imparted by high EPS production. As a result in our study even under water stress situation was prevailed under e-CO_2T, aerobic cultivars gave high yield with less plant stress enzymes production.

4.3. Soil enzyme activity

Soil enzymatic activities in rice rhizosphere are the mirror of microbial functioning, nutrient mobilization and water movement. This is not only an ecological indicator of rhizosphere functioning but also sensitive to elevated CO_2, temperature and moisture stress (Bhattacharyya et al., 2013, 2014,2016). Among soil enzymes dehydrogenase activity which dependent on the metabolic state of soil microorganisms was studied to assess biological activity of soil. We found higher dehydrogenase activity under e-CO_2T was due to higher organic matter, labile carbon contents in rhizosphere that lead to higher microbial activities in soil. Another important hydrolytic enzyme, fluorescein diacetate (3, 6-diacetyl fluorescein) was estimated to judge the potential activity of ester-cleaving enzymes that could be effectively used as indicator for microbial activity under e-CO_2T in rice (Schimel and Weintraub, 2003). In our study, FDA hydrolysis activity was increased significantly under e-CO_2T than ambient. Higher root exudation in e-CO_2T, enhanced root biomass carbon and increased soil labile carbon could be the probable reason (Saha et al., 2011; Bhattacharyya et al., 2013). Similarly, we found that e-CO_2T increased β-glucosidase activity (indicator of β-glycoside linkage break down of crop residues) in rice rhizosphere compared to a-CO_2 due to higher cellobiose availability and labile carbon content (Saha et al., 2011). All these higher activities of enzymes indicated greater rhizosphere activation which imparted addition intrinsic drought tolerance in aerobic system under elevated CO_2 and temperature.

4.4. Response of cultivars and crop growth stages under elevated CO2 and temperature

In our study aerobic rice cultivars and stages of crop growth significantly responded to elevated CO_2 and temperature. We found colloidal EPS, labile soil carbon (MBC, RMC) and soil enzymatic activities (dehydrogenase, FDA, and β-glucosidase) were significantly higher under CR Dhan201 compared to other two varieties tested. Those clearly showed its higher adaptability in anticipated climate change condition. Therefore, it could imparted relatively (compare to other two cultivar tested) higher intrinsic draught tolerance to aerobic system. In this connection it is important to note that the variety, CR Dhan201 was specifically released for aerobic ecologies (Das et al., 2015) by using APO is one of the parents. This showed an another possibilities to breed resistant aerobic varieties for anticipated climate change condition by considering the traits and condition quantified in our study.

Invariably soil carbon pools and enzymatic activities were found significantly higher at PI stage of crop growth which was corroborating our previous finding (Bhattacharyya et al., 2013, 2014, 2016). This observation clearly indicated that the PI is the most physiological active growth stage in respect of microbial functioning and nutrient mobilization. However, we got higher colloidal EPS in early vegetative stage (AT) of crop growth. The reason for that need to be investigates further in future study.

4.5. Plant stress enzyme

Additional water stress to aerobic rice affects plant–water potential that interfere the normal plant functions and also affect physiological and morphological traits (Rahdari and Hoseini, 2012). In order to overcome these additional water stress situation, plants produced different stress enzymes include superoxide dismutase (SOD), catalase and peroxidase (Sharma and Dubey, 2005). We maintained moisture stress condition (-40 kPa) both under a-CO_2 and e-CO_2T. It was observed, that EPS producing bacterial population as well as total soil EPS was higher under e-CO_2T that might enhanced the water holding capacity of soil (Vurukonda et al., 2016). Therefore, although the water stress condition was there, it was somewhat rectified under e-CO_2T. Further, we also noticed relatively less amount of plant stress enzyme activity under e-CO_2T than a-CO_2. Therefore, we conclude that e-CO_2T imparted additional drought tolerance in aerobic rice system.

4.6. Yield

Elevated CO_2 and temperature enhanced the rice yield in wet season in tropical India due to high biomass, photosynthates and C-allocation (Roy et al., 2012) provided there is no limitation of water and nutrient supply (particular N and P). We found yield increase under e-CO_2T in adequate nutrient supply even under moisture stress condition. Higher water retention in rice rhizosphere for longer time by EPS under elevated CO_2T could be the probable reason for that. So we can say this high yield of rice was due to additional drought tolerance imparted to the aerobic system under moisture stress by CO_2 elevation.

5. Conclusion

We found higher production of both total as well as microbial EPS (colloidal EPS) under e-CO_2T in the rhizosphere of aerobic rice cultivars which imparted additional intrinsic drought tolerance to the system. In anticipated climate change scenario, elevated CO_2 and temperature (2–3 °C rise) could impart additional drought tolerance to aerobic rice. There positive impact would be manifested by enhanced soil water holding capacity by producing higher EPS and better mineralization of nutrients in rhizosphere through enhanced labile carbon allocation and enzymatic activities in rice in general and aerobic rice in particular.

Acknowledgements

Part of the work is of MSc thesis of S Nayak. We acknowledge Director, ICAR-NRRI and NICRA for providing support to conduct the research works.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agee.2018.08.009.

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